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CONIDIAL FORMATION IN SPHAERONEMA FIMBRIATUM¹

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(WITH PLATE 7, CONTAINING 19 FIGURES)

During the past two years, a study has been made of the morphology and development of *Sphaeronema fimbriatum* (Ellis & Hark.) Sacc., a fungus causing a black-rot disease of the sweet potato (*Ipomoea batatas*). This organism is known to give rise to propagative bodies of several types, one of which is designated as hyaline conidia, another as olive conidia. The accounts by previous investigators are not entirely concordant, and certain of them appear to inadequately describe and interpret the formation of these structures. The purpose of this paper is, therefore, to set forth in detail the manner of formation of these two types of conidia and to compare these structures with the so-called endoconidia of *Thielavia basicola* Zopf.

While it is entirely beyond the scope of this article to review the literature on endoconidial formation in fungi, a proper understanding of the present problem necessitates a summary of the most important investigations on *Sphaeronema* and *Thielavia*. According to the account of Halsted and Fairchild (1), who first made a detailed study of *Sphaeronema fimbriatum*, the protoplast of the conidiophore which produces hyaline conidia ruptures or absorbs the apical wall of the conidiophore. A septum is then formed below the mouth of the conidiophore, thus cutting off the end of the protoplast to form a conidium. This conidium is pushed beyond the mouth of the conidiophore by growth of

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the protoplast behind it, and a number of other conidia are formed successively in the same manner. The mode of formation of the olive conidia differs from that of the hyaline conidia only in that they are not produced so rapidly, and in such other respects as are made necessary by differences in size and shape of the conidia.

Taubenhaus (2), in his studies of sweet potato black rots, states that spore formation in *Sphaeronema fimbriatum* resembles that in *Thielavia* in that "the spores are borne within the sheath of a terminal cell, and these are pushed out from within."

Concerning the manner of spore formation in *Thielavia basicola*, Zopf (3) states that the conidia of *Thielavia basicola* are formed in acropetal succession on short, several-celled conidiophores. The wall of each conidium becomes differentiated into two lamellae, the inner of which becomes the lateral wall of the conidium and the outer, the sheath out of which the conidia pass successively.

Gilbert (5) differing from Zopf's view, applies the term "endoconidia" to the spores of *Thielavia basicola* which "originate within the terminal cell and are not formed by its direct septation."

Brierley's (4) conclusions from a study of conidial formation in *Thielavia basicola* are at variance with both of these accounts. He affirms that the conidia are not formed endosporously within an endoconidial cell, but are acrogenously abjoined from the conidiophore. The first conidium is then liberated by a tangential splitting of its walls, which thus become differentiated into an outer closed sheath and an internal spore membrane. This outer sheath then ruptures, freeing the first conidium. Conidia subsequently produced are formed in the rear of the first in a manner in all respects like that of the first. Brierley further states that the process of conidial formation as he has described it for *Thielavia*, is probably that of all other "endoconidia" in fungi.

METHODS

In order to facilitate the observations recorded in this study, *Sphaeronema fimbriatum* was grown upon various substrata.

For part of the work, the fungus was grown on both cooked and uncooked sweet potato tissue for periods varying from thirty-six hours to six days. Material was then fixed in weak chromo-acetic solution (Schaffner's formula), dehydrated, imbedded in paraffin, sectioned 3 to 5 μ thick and stained either with Flemming's triple stain or Haidenhain's iron-alum haematoxylon. Mycelial mats grown on sterilized cornmeal as a substratum were likewise fixed, sectioned, and stained. For the study of living specimens, material grown on a substratum of 10 gr. wheat flour and 40 c.c. of water was especially favorable because of the unusually large size of the conidiophores. Very good differentiation was obtained when living conidiophores were stained for a few minutes in a one per cent. solution of safranin in fifty per cent. alcohol. Many observations were also made on living unstained material. The course of development of the two types of sporophores was followed by observations on the fungus cultured in Van Tieghem cells.

THE CONIDIOPHORE

Short spore-bearing branches, termed by Halsted and Fairchild (1) the primary sporophores, arise in great numbers from both aerial and subsurface hyphae. These primary sporophores are produced singly or in clusters of as many as six. When formed in clusters, the younger sporophore arises in order from a basal cell of the next older (Fig. 2). These structures are typically somewhat fusiform and usually consist of a basal portion of one to four short, more or less barrel-shaped cells with a long, tapering terminal cell which gives rise to the hyaline conidia. The primary sporophores vary in length, for the most part, from 70 to 100 μ and measure 4.5 to 6.5 μ at the thickest place. The terminal cell of the primary sporophore is herein designated the conidiophore. The conidiophore commonly has a length equal to or greater than one half the total length of the primary sporophore and the tip is usually narrower by 1 to 1.5 μ than the bulbous basal portion.

CONIDIAL FORMATION

At the initiation of the formation of the hyaline conidia, the distal end of the conidiophore is filled with protoplasm of a high refractive index, and contains a number of oil globules (Fig. 11) and sometimes small vacuoles. The basal portion may be occupied by a large vacuole surrounded by a thin layer of cytoplasm (Fig. 11). Shortly after a conidiophore has reached mature size, examination with great magnification ($\times 2270$) shows that its apical portion contains a fully formed conidium which has a distinct wall. This conidium is separated from the protoplast and wall of the conidiophore by a distinct line in such a manner that the wall of the conidiophore appears as a closed sheath about the conidium (Figs. 8, 9). This line of separation is visible when living specimens have been stained with safranin and the protoplast has been slightly plasmolyzed. When preparations are made in this manner, the protoplast takes the stain and the wall of the conidium and of the conidiophore remains clear. When the protoplast has not been plasmolyzed, however, the lateral conidial wall is pressed so closely against its sheath as to make the line of separation entirely invisible (Fig. 10). However, the line delimiting the basal wall of the conidium from the wall clothing the end of the protoplast of the conidiophore can at this time be discerned plainly. At each end of this line, a wedge-shaped opening caused by the convexity of the end of the protoplast and of the opposed newly formed conidium, is clearly seen. The size of this space depends upon the pressure exerted by the growing protoplast against the conidium above. When the pressure is great, the end of the protoplast is flattened against that of the conidium and the wedge-shaped space reduced (Fig. 8-10). In case the line separating the conidium from its sheath was visible, it is represented by a solid line (Figs. 8-10), otherwise, by a broken line. Other conidiophores are not uncommonly found whose distal ends contain a second fully formed conidium just below the first (Fig. 8). No more than two conidia within the sheath, however, have been observed. Careful examination of the free end of the sheath containing these conidia (Figs. 8, 9) shows that it is still intact and that the two conidia

have been formed acrogenously within the closed cell. The wall of the conidium is nearly, if not fully, as thick as that of the sheath surrounding it. This sheath does not appear thinner than the wall of the apical portion of the conidiophore before conidia had formed within it, nor yet less thick than the wall of the basal portion of the conidiophore.

After the formation of the second conidium within the conidiophore, or sometimes after the formation of the first, the apex of the sheath is dissolved and the conidia within are pushed out by growth of the protoplast behind them (Figs. 3, 5, 10). The fact that the opening at the end of the sheath is always bounded by a smooth line indicates that the tip has been dissolved; for if it were forcibly ruptured, the end should appear torn and broken. As soon as the protoplast has sufficiently elongated (Fig. 4), the next conidium is delimited and pushed out by continued growth of the protoplast. This process is repeated a great many times in succession, so that the acrogenously formed conidia appear in long chains (Fig. 1). Chains of as many as fifty-nine conidia have been observed. These conidia are hyaline, elongated, and have rounded, or often very angular ends (Figs. 1, 3 and 5). They may have a uniformly hyaline content with a single vacuole at each end, or the ends may be filled with granular protoplasm, leaving a clear zone at the middle. When the protoplasm at the ends is granular, numerous oil droplets can be seen within it. These conidia sometimes burst upon being put into water, whereupon the granules exhibit a Brownian movement. This has often been noted within unbroken conidia. The diameter of conidia produced by the same conidiophore is very uniform, but considerable variation in length obtains. The first conidia produced are longest, the last are shortest and the intermediate ones intergrade between the two extremes.

The method of spore formation by which the olive conidia arise, differs in certain particulars from that of the hyaline conidia. The conidiophore is typically short and unicellular as shown in Figs. 13, 14, and 16. There are those, however, whose length is equal to that of the primary sporophores (Fig. 17) and they may resemble these in shape. When the conidiophore

is fully developed, the first evidence of conidial formation consists in the dissolution of its apical wall. This is followed by the protrusion of the protoplast (Fig. 14) which is surrounded by a thin membrane. The protoplast enlarges greatly and becomes more or less pyriform with the narrow portion fitting as a plug into the mouth of the conidiophore (Fig. 15-17). Meanwhile, a septum delimiting the first pear-shaped conidium is being formed a short distance back of the mouth. The continued growth of the protoplast behind this conidium forces it beyond the mouth of the conidiophore. The apical portion of the enlarging protoplast then becomes delimited, forming the second conidium in a manner entirely similar to the first.

Olive conidia vary greatly in size and shape (Figs. 13, 15-17). In case formation progresses rapidly so that each conidium is pushed beyond the mouth of the conidiophore before its wall thickens, pressure within the conidium causes it to assume an elliptical shape. On the contrary, when conidial formation is less rapid, and the wall of a conidium becomes rigid before it is entirely without the conidiophore, its original pyriform shape is retained.

When first formed, these conidia are hyaline, but within 48 hours become olive-brown. They have a granular protoplasm having a varying number of oil droplets, the reserve food (Figs. 15, 17). As the conidia become older, the brown wall becomes thick and resistant (Figs. 18, 19). When these are placed in 50 per cent. sulphuric acid solution, the outer heavy wall bursts and a very thin, hyaline-walled vesicle slips out. Within a few moments this ruptures, liberating the protoplasmic contents. Most olive conidia range in size from 12 to 16 by 8 to 12 μ , a few being larger or smaller. In old cultures the smaller conidia predominate.

DISCUSSION

Upon comparing the account of conidial formation given above with that for *Thielavia basicola* by Brierley (4), certain essential points of difference can be noted:

1. If the first conidium of *Sphaeronema fimbriatum* were produced, as in *Thielavia*, by direct septation of the conidiophore and

subsequently liberated by a tangential splitting of its walls to form an outer sheath, the thickness of the sheath, as well as of the wall of the conidium, should be half that of the wall of the conidiophore before conidial formation. Instead of this, one finds that the wall of the conidium is nearly, if not quite, as thick as that of the sheath surrounding it. The sheath does not appear thinner than the apical wall of the conidiophore before conidia formed within it, nor less thick than that of the basal portion of this cell (Figs. 8-11).

2. It is clearly seen from Figs. 8-11 that the end of the protoplast of the conidiophore is not naked but protected by a wall fully as thick as that about the conidium above it. It is believed that this wall extends for some distance back from the tip. In Fig. 6, at (b), the wall of the protoplast is clearly separated from the sheath. At (c) the wall presses so closely to the sheath that the line of separation cannot be distinguished although they appear separate again at (a) for a short distance. In Fig. 7, the protoplast is seen to have grown beyond the sheath for a considerable distance without having formed conidia. It is invested with a delicate wall which is separated from the sheath for almost one-half the length of the conidiophore. The fact that this protoplast clothes itself with a wall for a distance from its tip greater than the length of a conidium indicates that the conidia are not formed by direct septation of the conidiophore and that the sheath takes no part in the formation of the conidial wall. The septum cutting off each conidium must arise from the new wall with which the protoplast invests itself and not directly from the wall of the conidiophore.

3. In several instances conidia, as illustrated in Fig. 12, have been observed which had germinated prior to escape from the conidiophore. This indicates that conidia mature while still wholly within the sheath.

On the basis of the evidence presented and discussed above, in which conidial formation for *Sphaeronema* seems to differ from *Thielavia*, the first and often the second, hyaline conidium in a series can properly be regarded as an endoconidium. It is clearly delimited within a closed cell whose wall remains intact

until the conidium has reached maturity. However, conidia produced subsequently to the second, cannot be regarded as endoconidia, for while they are formed within a sheath, this sheath is open at the tip. In true endospore formation, the spores are delimited within a closed cell, although the spore walls may not develop until after escape of the spore masses.

It is apparent that the olive conidia are not endospores. They are formed by the swelling of the protoplast beyond the opening at the tip of the conidiophore and are not separated from the protoplast until the conidium has reached mature size.

SUMMARY

1. A study has been made of the formation of the hyaline and the olive-brown conidia of *Sphaeronema fimbriatum*.
2. Contrary to Brierley's anticipations, the process of conidial formation in *Sphaeronema* differs in certain particulars from that in *Thielavia*.
3. In the formation of the first conidium, the distal end of the protoplast of the conidiophore invests itself with a new wall and separates as a conidium.
4. This conidium is liberated by dissolution of the tip of the conidiophore.
5. Often a second conidium is formed in the same manner as the first and before dissolution of the tip of the conidiophore.
6. The first two conidia should be regarded as endoconidia.
7. Conidia produced subsequently to the second are not endoconidia.
8. Olive-brown conidia are not produced endosporously.

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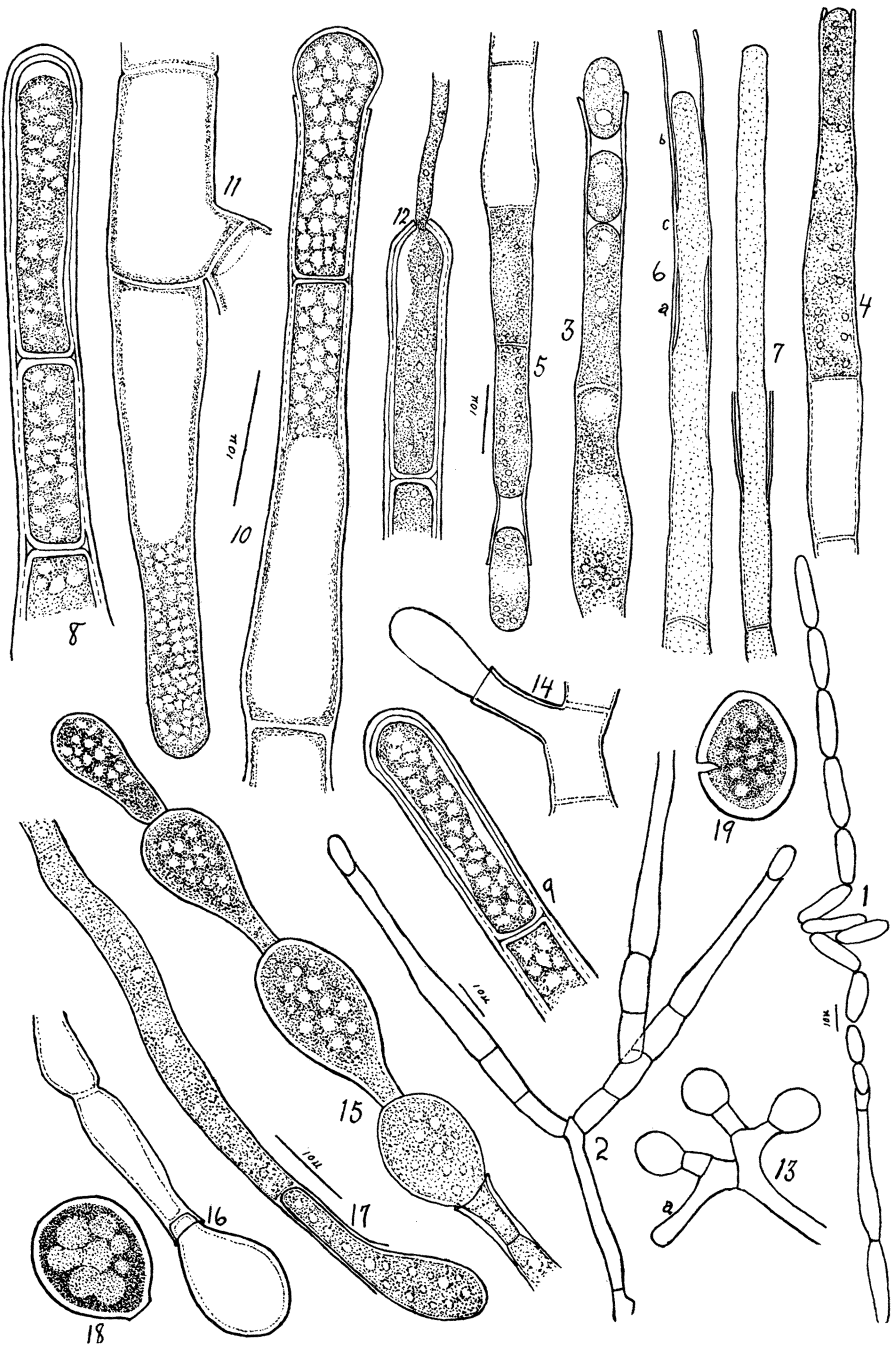
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EXPLANATION OF PLATE 7

All the drawings were outlined and as many as possible of the details put in with the aid of a camera lucida. A different magnification was used for each of the following groups of figures: 1; 2, 13; 3-7, 14-17; 8-12. The reduced magnification can easily be calculated from the scale given with each group.



SPHAERONEMA FIMBRIATUM

- Fig. 1. Conidiophore producing hyaline conidia.
- Fig. 2. Cluster of three conidiophores producing hyaline conidia.
- Figs. 3 and 5. Conidiophores with hyaline conidia escaping.
- Fig. 4. Conidiophore out of which all conidia have been forced by growth of the protoplast behind them preparatory to formation of other conidia.
- Figs. 6 and 7. Conidiophores. See text for explanation.
- Fig. 8. Distal end of a conidiophore showing two fully formed conidia within the unbroken sheath.
- Fig. 9. Similar to Fig. 8, but showing only one conidium within the sheath.
- Fig. 10. The tip of the conidiophore has been dissolved and the first-formed conidium is being slowly shoved out by growth of the protoplast behind it.
- Fig. 11. Conidiophore shortly before formation of the first conidium.
- Fig. 12. Conidium germinating while still within its sheath.
- Fig. 13. Group of conidiophores producing olive conidia.
- Fig. 14. Higher magnification of Fig. 13 (a) showing how the protoplast grows out to form the first olive conidium.
- Fig. 15. Chain of olive conidia showing variation in shape and size and how each conidium is delimited by a wall below the mouth of the conidiophore.
- Fig. 16. Conidiophore with an olive conidium plugging its mouth.
- Fig. 17. An unusually long conidiophore of this type. The conidium is completely delimited but has not assumed its final shape.
- Figs. 18, 19. Olive conidia with thick walls characteristic of mature spores.